AN ANGLE-TUNABLE MICROFLAP TOWARD THE OBSERVATION OF PARASITE INVASION INTO HOST ADHERENT CELLS

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ABSTRACT

In this paper, we demonstrate the angle-tunable microflap system for observing parasite invasion into adherent host cells. This system enables us to control the inclination angle of cell-culturing microflaps by applying external magnetic fields. A fabricated microflap consists of two materials, parylene and permalloy, and adherent host cells are patterned on the microflaps. We successfully kept adherent cells on the microflaps inclined by an external magnetic field, and observed boundary of cell membrane. Thus, we clearly observed how parasites invaded through cell membrane from multiple angles, and judged completed invasion. This method is widely applicable to the analysis of interaction between infectious microbes and host cells.

KEYWORDS

Parylene, Microflap, Parasite invasion.

INTRODUCTION

Cytological studies for infectious microorganism have recently garnered much attentions. Especially in infectious microbe research, it is important to observe the penetration and invasion of obligate intracellular pathogens such as bacteria or parasites, which gives us much information about morphological changes and expressed proteins [1]. By combining visualization of targeted proteins using genetic engineering techniques, we can identify the exact timing and location of expressed protein during attachment and invasion to host cells [2]. In the case of floating host cells such as leukocytes and erythrocytes, it is possible to detect the infecting microbes because the penetration process can be clearly observed between invading cells and host cells in suspension on the Petri dish under a microscope [3-4]. With conventional methods, however, it is difficult to capture pathogen invasion into two-dimensionally cultured adherent cells and to confirm whether parasites are located inside host cells or not, because the invasion direction and optical path for the observation are in the same axis (Figure 1). In this study, we propose the micro-fabricated flapping system to observe adherent cells in order to detect the boundary membrane of parasites and host cells as shown in Figure 1. The devices are able to respond to applied magnetic fields. Under the control of the magnetic field, the inclination angle can be controlled by applied magnetic field, and cells attaching on microflaps can be inclined and observed from the desired angles.

EXPERIMENT

We utilized parylene-C (DPX-C, Speedline Technology) as a material of microflaps. First, a 100-nm-thick Parylene layer was deposited on a glass substrate coated with an alginate sacrificial layer. A chromium layer and a 170-nm-thick

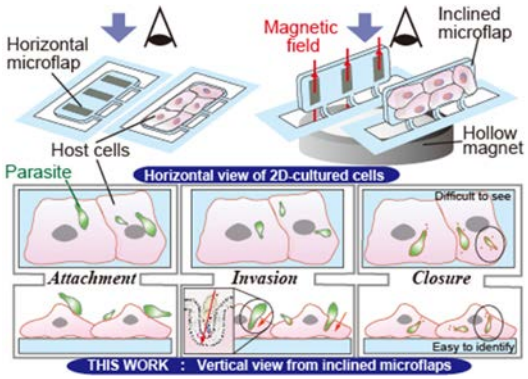

Figure 1: A schematic image of magnet-active microflaps for inclining cells. In conventional methods to observe adherent cells, it is difficult to observe and analyze cell-cell interactions between more than two cells such as cell-cell membrane contact or the infection of microbes because of the optical path. Microflaps for inclining cells up will make it easier to observe membrane contact areas between paired two cells.

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permalloy (78 Permalloy, Nilaco corp.) layer were sputtered. Permalloy layer generates the inclination torque. We patterned permalloy by aqua regia. A permalloy pattern was covered with parylene again, and a hinged microstructure was fabricated by O_2 plasma. Fibronectin and 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer were coated on the substrate to pattern cell-adhesive/non-adhesive area, respectively. After removing alginate layer, the microflaps were released from glass substrates and responded to magnetic field (Fig.2(b-c)).

In this study, HFF, human foreskin fibroblast cells, were cultured as host cells of *Toxoplasma gondii*. They were cultured in D10 medium supplemented with Dulbecco's modified Eagle medium, 10 mM HEPES, 10% fetal bovine serum, 2 mM glutamine and 10 μg/ml of gentamicin. *T. gondii* strain RH was maintained in HFF cells. They were propagated as tachyzoites in grown in D10 medium as shown in HFF cell culture. Parasites were separated from the host cells by the aspiration with 21G needle and the filtration through polycarbonate membrane filters (pore size 3 μm). They were washed with PBS buffer and centrifuged to eliminate host cells and cell debris. To test the magnetic response, we set up a microscope, a magnet on a micropositioner and gauss/tesla meter probe. A series of permanent NdFeB magnets (NeoMag Co., Ltd., Japan) were mounted onto the micropositioner, which provided the precise control of the magnet position. We observed cellular behavior of host cell and parasite invasion under an inverted microscope (IX71, Olympus, Japan).

RESULTS AND DISCUSSION

To investigate the effects of the external magnetic fields on inclination angles, we measured angles of the hinged microstructures. After adding the alginate lyase, microflaps are released from the substrates and inclination angle was measured under the control of external magnetic fields (Figure 2(a)). Inclination angles increased with increasing the external magnetic fields. We measured inclination angles of the hinged microstructures under different external magnetic fields. It can be clearly seen that inclination angles were different for the same magnetic field. Inclination angles also increased as hinges became longer, wider or the volume of permalloy became larger.

As a model of microbial infection, we used a species of parasites *Toxoplasma gondii* and their host HFF cells. First, we successfully cultured and patterned HFF cells onto microflaps. Cells are only attached to the Fibronectin-area and not attached to MPC polymer-coated area. After culturing cells onto microflaps, the sacrificial layer was removed and microflaps were released by adding alginate lyase (Figure 2(b)). The inclination angle could be easily controlled and adherent cells could be inclined (Figure 2(c)). Magnetic response of this device was independent of attaching cells and dependent upon cultured cells. During the microflap manipulation, cells remained attached to the surface of the microflaps and their cellular membrane could be clearly observed (Figure 2(d)). Figure 3 shows time-lapse images

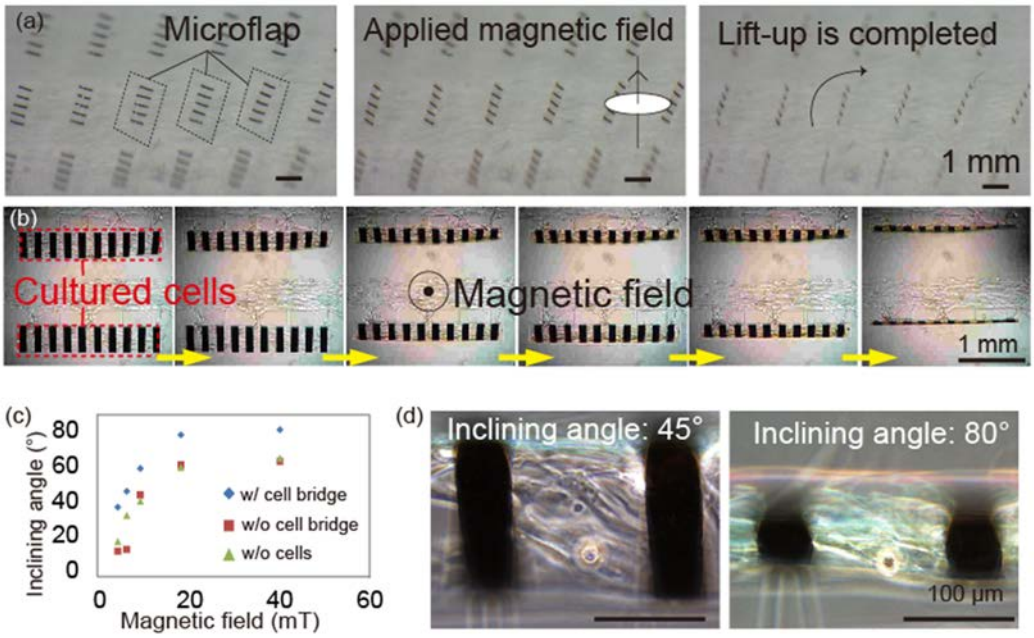

Figure 2: (a) Time-lapsed images of the motion of microflaps with permalloy. The relation between inclining angles and the strength of magnetic field in comparison with L_{mag} (a-1), W_h (a-2) and L_h (a-3). The microflap structure functions after sacrificed layers are dissolved by alginate lyase. (b) Flapping motions of microflap coated with adherent cells (HFF cells). By increasing the strength of magnetic fields, cells were inclined and lifted-up. (c) Inclination angle of microflaps with bridging cells or without cell was measured. (d) During the manipulation, cells remained attached to the surface of microflaps and cellular membrane could be clearly observed. By changing the inclination angle, we could observe cells from various angle.

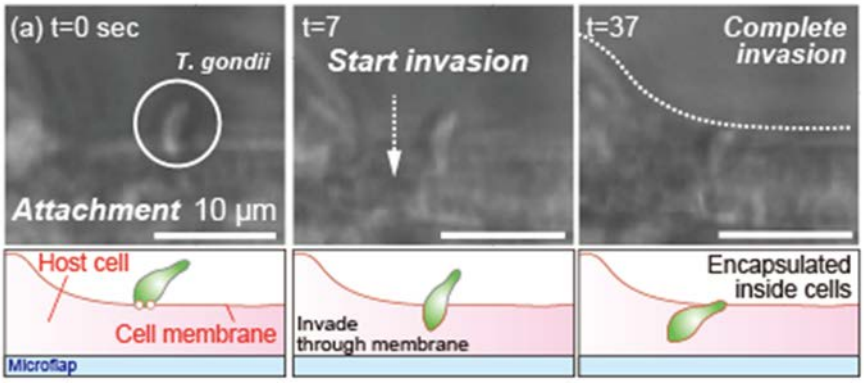

Figure 3: Time-lapse images of Toxoplasma gondii invasion into the host HFF cells. We could optimize the angle to observe cells and cellular membrane clearly. First, parasites attached to the cell membrane for about 7 sec, and then it took about 30 sec to invade HFF cells.

indicated that parasites totally invaded. After focusing on HFF cells which parasites approached to, we made microflaps inclined in the desired orientation. We could optimize the angle of cells and obtain the image of membrane contact area and invasion time-scale of real-time movies.

CONCLUSION

In this study, we proposed the inclination technology to manipulate adherent host cells for the analysis of parasite invasion. We fabricated a hinged microflap device encapsulating permalloy thin layer with magnetic-field response. Host cells were able to adhere onto the surface of microflaps and stretch around fibronectin-coated area. This device allowed us to change the inclination angle of cell-adherent surface, capture the penetration of *Toxoplasma gondii* into host cells and successfully observe time-lapse behavior before/after invasion. As well as *Toxoplasma gondii*, this observation technique can be widely applied to understand the mechanism of invasion of various types of other obligate intracellular parasite, virus and bacteria.

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